CHAPTER 2

Alphavirus Structure

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I. INTRODUCTION

Alphavirus particles are roughly spherical, with three principal substructures—an outer glycoprotein shell, a lipid bilayer, and an RNA-containing core (nucleocapsid). The radial organization of these components is summarized in Fig. 1. The most extensive structural studies have been carried out on Sindbis virus and Semliki Forest virus (SFV). It is on these very similar viruses that the description below will focus.

II. GENERAL PROPERTIES

The following characteristics of the substructures are well established: (1) The glycoproteins are arranged in an icosahedral surface lattice with 240 structure units per particle—a so-called $T = 4$ lattice (von Bonsdorff and Harrison, 1975, 1978; Adrian et al., 1984). The lipid bilayer, derived from the cell membrane during budding, contains a representative sample of cellular plasma membrane lipids (Laine et al., 1972; Hirschberg and Robbins, 1974). Hydrophobic sequences near the C termini of the E1 and E2 polypeptide chains form structures that cross the bilayer (Garoff and Simons, 1974; Garoff and Söderlund, 1978; Rice et al., 1982). (3) The core is an isometric particle, with a radius of about 200 Å. It probably has icosahedral symmetry, although the details of its surface organization are not certain. There is, however, reason to believe that like the glycoprotein shell, it has a $T = 4$ lattice and 240 subunits.

Microcrystals of SFV have been observed in the electron microscope, and the packing of virions in these lattices has been analyzed by Wiley.
FIGURE 1. Organization of protein and lipid in Sindbis virus particles. Semliki Forest virus is essentially identical, but about 10 Å smaller in diameter. The pear-shaped units represent (E1 + E2) heterodimers. Their clustering in trimers gives the grooved patterns seen in electron micrographs. The hydrophobic C-terminal roots of E1 and E2 penetrate the lipid bilayer. Those of E2 have a small internal domain, shown here making contact with a site on a nucleocapsid subunit. The bilayer is symbolized in the diagram by an array of lipid molecules between radii of 210 and 255 Å. The icosahedral surface lattices that characterize the outer glycoprotein layer and the inner core are shown in the lower part of the drawing.

and von Bonsdorff [1978]. Larger crystals, giving weak low-resolution X-ray diffraction, have also been obtained [F. K. Winkler, personal communication]. Crystallization shows that the virus particle is a precise and regular structure and that all particles are identical.

A. Radial Organization

Small X-ray scattering from Sindbis virus and SFV [Fig. 2] shows the position of the lipid bilayer and the radial dimensions of the core and of
FIGURE 2. Electron densities, as functions of radius, for Sindbis virus [Harrison et al., 1971] and SFV [Harrison and Kääriäinen, unpublished data], as determined by small-angle X-ray scattering. The deep trough shows the position of the lipid bilayer.

the glycoprotein coat. The SFV particle appears to be slightly smaller than that of Sindbis virus, but there is otherwise no fundamental difference. The lipid content is sufficient to make essentially continuous bilayers with head groups occupying shells at the indicated radii [Harrison et al., 1971; Laine et al., 1972]. The area of the outer leaflet is about 40% larger than that of the inner leaflet, imparting a substantial asymmetry.

B. Glycoprotein Shell

Figures 3–5 show electron micrographs of Sindbis virus. The principal contrast comes from the arrangement of glycoprotein in the outer shell. When negatively stained with phosphotungstate [Fig. 3a–e], the virus appears to be significantly flattened, and most of the image comes from one side of the particle. Stain penetrates along grooves in the surface and at nodes where five or six grooves meet. The grooves delineate a
FIGURE 3. (a–c) Selected images of Sindbis virus particles negatively stained with potassium phosphotungstate. Each particle is shown twice, with fivefold nodes of the surface lattice marked by stars on the lower image. (c–c) These images show two or more fivefold positions, in a relationship corresponding to a T = 4 lattice. (f) Field of Sindbis particles negatively stained with uranyl acetate. The particles are less deformed than in potassium phosphotungstate. Scale bars: 1000 Å. From von Bonsdorff and Harrison [1975].
T = 4 icosahedral surface lattice. The characteristic pattern for such a lattice is a sequence 5-6-6 for the coordination of nodes along a lattice line. Several images displaying this criterion are included in Fig. 3. Negative staining in uranyl acetate preserves the particle somewhat better (Fig. 3f), but two-sided contrast often makes the images less clearly interpretable.

Recent advances have yielded images of unstained SFV [Adrian et al., 1984] and of Sindbis virus [Fig. 4] using direct transmission microscopy of specimens in amorphous ice at liquid N₂ temperatures. The virus particles are much better preserved than in negative stain, and the T = 4 icosahedral surface lattice is clearly revealed in particles viewed along appropriate symmetry axes. The complete superposition of contrast through the entire particle makes other views appear confusing on subjective inspection, although the preservation of detail is in fact equally good.

Shadowing of frozen specimens, uncovered by deep-etching after cleavage, gives a dramatic view of the surface topography of Sindbis virus [Fig. 5] (von Bonsdorff and Harrison, 1978). The pattern of fivefold and sixfold nodes, characteristic of a T = 4 lattice, is again present.

The glycoproteins of Sindbis virus and SFV are thus associated into roughly triangular clusters in a T = 4 icosahedral lattice on the surface of the alphavirus particle. One such cluster consists of three (E₁ + E₂) or (E₁ + E₂ + E₃) units, grouped about a local threefold symmetry axis. Further evidence for the organization of these trimer groups comes from analysis of hexagonal glycoprotein arrays, which are described in Section II.E. The local association of E₁ and E₂ as heterodimers in virions and in Triton-solubilized glycoprotein has been demonstrated by chemical cross-linking, both in SFV (Ziemiecki and Garoff, 1978) and in Sindbis
virus [Rice and Strauss, 1982]. In the case of SFV, it is plausible to suppose that one copy of E3 is associated with each E1--E2 heterodimer, although no cross-linking is actually observed [Ziemiecki and Garoff, 1978]. The cross-linking of intact Sindbis virus not only shows that E1 and E2 are closely associated, but also demonstrates very clearly that three such heterodimers form a cross-linkable cluster in the viral surface [Rice and Strauss, 1982]. This clustering does not survive Triton X-100 treatment, since species larger than the heterodimer are not observed when cross-linking detergent-solubilized glycoprotein. The chemical results are thus in complete agreement with the interpretation of micrographs given above. Assuming that all sites in the lattice are occupied [vacant sites are not seen in electron micrographs], the virion must contain 240 copies of each glycoprotein chain.

Gahmberg et al. [1972] and Utermann and Simons [1974] first used proteolytic cleavage of SFV to show that small portions of E1 and E2 lie buried in the lipid bilayer. Subsequent cross-linking experiments indicated that E2 does indeed interact directly with the core [Garoff and Simons, 1974]. Analysis of the amino acid sequences of SFV and Sindbis virus glycoproteins, derived from sequences of complementary DNA, shows probable transmembrane segments near the C termini of both E1 and E2 [Garoff et al., 1980a; Rice and Strauss, 1981]. It is likely that these hydrophobic sequences form α-helices, since they are of suitable length.
to span a bilayer as a single helix. The only internal part of E1 is the dipeptide Arg-Arg. A larger segment of E2 (33 residues in Sindbis virus, 31 residues in SFV) faces the core, presumably interacting with a binding site on its surface (Rice et al., 1982). The sequence of this segment is reasonably well conserved between Sindbis virus and SFV, as is the non-basic, C-terminal domain of the core polypeptide. The glycoprotein domain organization is summarized in Fig. 6.

Details of the glycoprotein structure at high resolution are not yet known. Some inferences may be made by analogy with the influenza virus glycoproteins, the structures of which have been determined by X-ray crystallography (Wilson et al., 1981; Varghese et al., 1983). As in the influenza virus proteins, E1 and E2 extend more than 100 Å outward from the membrane surface. Oligosaccharides (two each on E1 and E2 of Sindbis virus, one on E1 and two on E2 of SFV) are significantly "buried" in the virus, as measured by the ratio of glycosidase sensitivity of intact virus to sensitivity of detergent-solubilized glycoprotein (McCarthy and Harrison, 1977). This result suggests that as in the influenza virus proteins, some of the glycosylation sites are on lateral surfaces of the projecting protein, rather than at the outer tip. A low-pH-induced fusion activity in SFV (Helenius et al., 1980) implies that a conformational change in one or both of the major glycoproteins may reveal a sequence or structure that facilitates fusion of viral membranes with cell membranes to which they are bound (see Chapter 4). There is evidence that in influenza virus hemagglutinin, a dramatic conformational change occurs at low pH (Skehel et al., 1982), exposing a "fusion peptide" that is normally buried in the folded structure.
FIGURE 7. Schematic section through the middle of the lipid bilayer in Sindbis virus or SFV. The transmembrane segments of E1 and E2 are represented as large circles, with the van der Waals radius of an α-helix in cross sections. Each small solid circle represents one phospholipid and one cholesterol head group. The molar ratio of phospholipid and cholesterol in SFV is about 1:1 [Laine et al., 1973], and the area of a phospholipid-cholesterol pair is taken as 90 Å² [Rand and Luzzati, 1968].

C. Lipid Bilayer

The lipid composition of alphaviruses represents rather closely a random sample of plasma membrane lipids in the cells in which they are grown [Laine et al., 1973]. As in most enveloped viruses, there is little evidence for strong preferential exclusion or inclusion of any particular class of lipids, and by growing virus in different host cells, appropriate variation of viral lipid composition is obtained [Luukkonen et al., 1976]. The difference in areas of inner and outer leaflets may generate some overall compositional differences, due to the asymmetry of the cell membrane itself. An asymmetry in composition of the SFV bilayer has been shown by van Meer et al. (1981). Figure 7 shows a schematic cross section through the bilayer, indicating the relative areas occupied by lipid mol-
ecules [taken as equimolar phospholipid and cholesterol [Laine et al., 1973]] and by transmembrane segments of the glycoprotein "roots" [assumed to be α-helical]. The figure was drawn assuming a surface area of about 90 Å² for a phospholipid–cholesterol pair [Rand and Luzzati, 1968]. We do not know whether or not the transmembrane segments of E1 and E2 are closely paired (forming for example, an α-helical coiled coil), but they are drawn here assuming some interaction.

D. Core (Nucleocapsid)

The cores of Sindbis virus and SFV may be isolated by gentle detergent treatment of virus particles. When negatively stained with uranyl acetate, cores appear in the electron microscope as spherical particles, about 400 Å in diameter. The lack of striking surface features has prevented definitive analysis of the surface lattice. The reported chemical composition of both viruses and cores is consistent with the presence of either 240 core subunits [a T = 4 lattice] or 180 subunits [T = 3]. An icosahedral arrangement congruent with the glycoprotein surface lattice is a plausible structure, since one-to-one interaction of E2 "roots" with core subunits accounts in a simple way for specificity in budding [see below]. Better micrographs, taken of unstained cores embedded in vitreous ice, will probably resolve the issue.

The amino acid sequences of Sindbis virus [Rice and Strauss, 1981] and SFV [Garoff et al., 1980b] core proteins suggest a modular organization, similar to that of a number of plant-virus coat proteins [Harrison, 1983, 1984a,b]. The N-terminal regions—about 115 residues in Sindbis virus and SFV—have sufficient positive charge to neutralize over half the approximately 55 RNA phosphates per subunit. These parts show moderate sequence similarity between the two viruses, and they appear to correspond to the flexibly tethered, inward-projecting "R domains" of icosahedral plant viruses such as tomato bushy stunt virus [Hopper et al., 1984; Harrison, 1984b]. There is a striking frequency of proline, as well as of lysine and arginine, in this part of the chain. The remainder of the core protein sequences, about 150 residues, do not have strong positive charges, but they do show very marked conservation between Sindbis virus and SFV. They probably correspond structurally to the parts of the subunits that form the rigid shell of the core and that interact with the internal peptide of E2.

The RNA of spherical plant viruses has been shown to be tightly condensed within a cavity defined by the inner surfaces of coat subunits. The RNA retains most or all of its secondary structure, and the helical stems may be locally well packed against each other. There is no fixed geometry of interaction with the rigid part of the coat subunit, and RNA segments bind principally to flexibly linked, N-terminal R domains. It is
likely that this description also applies to the RNA in Sindbis virus and SFV cores.

The cores of SFV shrink about 20% in diameter when exposed to low pH [von Bonsdorff, 1973]. The mechanism of this transition is not clear. Comparable treatment of Sindbis nucleocapsids [pH 6 or lower] appears to lead to dissociation [Harrison, unpublished observation].

E. Hexagonal Glycoprotein Arrays

Treatment of SFV and Sindbis virus with increasing quantities of Triton X-100 produces the different stages of virion disruption that are diagrammed in Fig. 8 [Helenius and Söderlund, 1973]. Detergent adds to the bilayer, causing viral membranes to expand and ultimately to fuse. Cores appear to be expelled in the process. In the case of Sindbis virus, glycoproteins in the fused membrane vesicles can be induced to crystallize under suitable conditions, forming two-dimensional hexagonal arrays [see Fig. 5]. The arrangement of glycoprotein in these arrays is similar to that found in the intact particles. The [E1 + E2] units are clustered in trimers, with six trimers grouped around each sixfold axis of the hexagonal unit cell. Identical trimers are found in the surface of the virus particle, grouped in sixes and in fives, and in that case, the fivefold groupings generate the curvature of the icosahedral surface lattice.

Formation of extensive arrays, such as shown in Fig. 8, on the surface of an infected cell might be expected to inhibit budding, since formation of fivefold vertices would require removal of a large wedge of protein. It is therefore unlikely that such arrays represent an intermediate stage in viral assembly. Their spontaneous formation does indicate, however, precise and reasonably strong glycoprotein–glycoprotein contacts.
III. VIRION ASSEMBLY

Assembly of alphaviruses is compartmentalized. Nucleocapsids form in the cytoplasm from 49 S RNA and core subunits, and the completed nucleocapsids acquire lipid and glycoprotein by budding out through the cell membrane. Host membrane proteins are efficiently excluded, presumably by the close packing of glycoproteins in the regular surface lattice.

What drives the process of viral budding? Budding involves formation of contacts between E2 cytoplasmic domains and presumptive binding sites on the nucleocapsid (see Fig. 1), as well as acquisition of glycoprotein interactions in the surface lattice. The glycoproteins tend by themselves to form hexagonal arrays, as just described, so the icosahedral geometry of the surface lattice appears to be determined by the symmetry and curvature of the nucleocapsid, rather than by glycoprotein interactions alone. There is no evidence, either morphological or biochemical, that host-cell factors or metabolic energy are required in the process (see Simons and Warren, 1984). The following self-assembly picture therefore seems to be the simplest description of likely events at the plasma membrane: A cluster of glycoproteins interacts, via a set of internal domains, with a nucleocapsid. The strongest lateral interactions are between E1 and E2 in heterodimers, and there are also strong contacts within trimes of these (E1 + E2) units. It is therefore possible that a trimer of heterodimers, presenting three internal domains to the nucleocapsid, can form a contact with the core sufficiently stable to initiate assembly. Recruitment of further glycoprotein units causes the membrane to wrap around the nucleocapsid. Addition of these units is stabilized by contacts between the E2 internal domain and the core. If the core is a 240 subunit, T = 3 structure, these contacts are identical to the initial one. If the core is a T = 3 particle, however, then not all the E2 internal domains can bind to identical sites. Some flexibility of the connection between external domains and transmembrane segments—or of the internal domains themselves—could allow a significant number of the E2 “roots” to contact sites on the core, with the remaining glycoprotein units held in the particle by lateral interactions such as those found in hexagonal arrays. The final pinching off may not require any additional mechanism, since the strain on the membrane could be sufficiently great to cause the bilayer to close around the nucleocapsid as the last glycoproteins add to the growing shell. There is, however, no definite evidence on this point.

Nucleocapsid assembly is probably analogous to assembly of simple RNA viruses, such as tomato bushy stunt and turnip crinkle viruses (Harrison, 1983; Sorger et al., 1986). In these cases, an initiation complex, composed of a defined cluster of subunits and a particular RNA “packaging sequence,” appears to determine specific incorporation of viral RNA. Assembly proceeds by incorporation of coat subunit dimers, and
interactions of N-terminal arms determine which of two conformations a dimer will adopt when it adds to the growing shell (Harrison, 1984a,b; Sorger et al., 1986). Reassembly of Sindbis virus cores in vitro (Wengler et al., 1982) shows that in this case also, core subunits and viral RNA are sufficient to determine correct assembly and that no further components are required. A specific model for \( T = 4 \) self-assembly has been outlined (Harrison, 1983). It involves addition of subunit trimers to an appropriate nucleus. It postulates two conformations for the trimer, with 20 trimers in one state and 60 in the other, and it involves, as in \( T = 3 \) self-assembly, an interaction (e.g., of N-terminal arms) that correctly determines which conformations a trimer will adopt when incorporated into the structure.

To demonstrate that the mechanisms suggested herein are correct, it is clearly important to establish conclusively the structure of the core and to examine directly the postulated interaction between the E2 internal domain and the nucleocapsid. Indeed, only by obtaining budding in vitro of purified components will it be possible to rule out participation of other factors in the assembly of the viral membrane.

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REFERENCES


